REMARKS

This amendment is in response to the Office Action, dated August 6, 2009 ("Office Action"). Claims 17-28 remain pending. No new matter has been added.

35 U.S.C. §103(a)

Examiner rejected claims 17-28 under 35 U.S.C. §103(a) as being obvious over Fan, *et al.*, in view of Oka, *et al.* and Sharifi, *et al.*

Examiner asserts that Fan, *et al.* teaches the AAV-based delivery of human atherosclerosis A-I into the skeletal muscle for the treatment of atherosclerosis, but does not teach the Apo A-I gene as the Apo A-I Milano gene or where the administration comprises delivery to bone marrow cells. Oka, *et al.* teaches the administration of AAV particles expressing Apo A-I Milano gene for the treatment of atherosclerosis, and Sharifi, *et al.* teaches adeno-associated virus mediated Apo A-I Milano gene therapy for the treatment of atherosclerosis and restenosis. One of skill in the art, Examiner asserts, would have been motivated to modify the teachings of Fan, *et al.* with the teachings of Oka, *et al.* and Sharifi, *et al.* because Oka, *et al.* teaches that Apo-AI Milano was demonstrated to have a greater anti-atherogenic effect than that observed for wild-type Apo-AI.

In response, Applicants submit that Oka, *et al.* was published less than a year before the priority date of the present application and describes the work of the inventors. Oka, *et al.* highlights various abstracts of the 2003 annual meeting of the American Society of Gene Therapy (ASGT), including an abstract described as "Patel, *et al.* (City of Hope National Medical Center, Duarte, CA)." Applicants submit that the relevant disclosure of Oka, *et al.* is in fact a summary of the Patel, *et al.* reference, co-authored by Applicants, and enclosed herein as Exhibit A, and in an Information Disclosure Statement submitted herewith.

Applicants submit that to the extent that the instant invention is described in the Patel, *et al.* reference, it describes solely the work of Applicants. (*See* Declaration of Prediman K. Shah Pursuant to 37 CFR 1.132, Section 3). Additionally, with regard to the Oka, *et al.* reference, Applicants submit that conception of the invention as claimed was completed prior to the publication of the Oka, *et al.* reference. (*See* Declaration of Prediman K. Shah Pursuant to 37 CFR 1.131, Section 5). Prior to the publication of the Oka, *et al.* reference, Applicants tested anti-atherogenic properties of both transplantation with ApoA-1 Milano transduced bone marrow

cells as well as direct intramuscular injection of rAAV-2 and rAAV-5 vectors encoding ApoA-I Milano. (*See* Declaration of Prediman K. Shah Pursuant to 37 CFR 1.131, Section 6). Between May 2003 and April 2004, Applicants conducted additional studies to generate further support for the long term inhibition of atherogenesis and efficacy of treatment. (*See* Declaration of Prediman K. Shah Pursuant to 37 CFR 1.131, Section 7). From February 2004 to April 2004, Applicants worked with patent counsel and shortly thereafter constructively reduced to practice the invention as claimed by filing the application to which the present application makes a claim of priority. (*See* Declaration of Prediman K. Shah Pursuant to 37 CFR 1.131, Section 8). Thus, because conception of the invention as claimed was completed by Applicants prior to the publication of Oka, *et al.*, followed by diligent efforts by Applicants to reduce the invention to practice, Applicants submit that the rejection of claims 17-28 under 35 U.S.C. §103(a) as being obvious over Fan, *et al.* in view of Oka, *et al.* and Sharifi, *et al.* should be withdrawn, as the cited references are not prior art.

All of the claims in the application are now believed to be allowable. Favorable consideration and a Notice of Allowance are earnestly solicited. If for any reason Examiner finds the application other than in condition for allowance, Examiner is requested to call either of the undersigned attorneys at the Los Angeles telephone number (213) 633-6800 to discuss the steps necessary for placing the application in condition for allowance.

Respectfully submitted,
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55. rAAV-Mediated Apolipoprotein A-1 Milano Gene Therapy for Atherosclerosis

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ApolipoproteinA-1 (ApoA-1), a major component of high density lipoprotein (HDL), has been shown to have anti-atherogenic properties. Recently, an Arg173 to Cys point mutation, ApoA-1 Milano, has demonstrated efficacy in both the prevention and treatment of atherosclerotic lesions in murine and rabbit animal models, with potency greater than that of wild type ApoA-1. We tested the anti-atherogenic properties of rAAV-2 and rAAV-5 vectors encoding ApoA-1 Milano in homozygous transgenic ApoE-/- mice which develop large vessel atherosclerotic plaques when fed a high fat diet. Foam cells, which play a pivotal role in atherogenesis, arise from bone marrow-derived macrophages, and transplantation with wild type bone marrow cells has been shown to prevent the onset of atherosclerosis in ApoE-/- mice. In this study we tested the hypothesis that transplantation of transduced bone marrow cells would protect against the development of atherosclerosis. The efficacy of transplantation with ApoA-1 Milano transduced bone marrow cells in reducing the extent of atherosclerosis was compared with direct intramuscular vector injection. The latter results in secretion of ApoA-1 Milano directly into the circulation. For the transplants, ten million ApoE-/- bone marrow cells were transduced overnight with rAAV-ApoA-1 Milano at mpi: 5000, washed and infused via the tail vein into 6-8 week old, lethally irradiated male Apo E-/- mice. For the intramuscular injections, 6-8 week old ApoE-/- mice were injected with $3\text{-}5\times10^{12}$ vector genomes/kg. A high fat diet was initiated two weeks after the procedures. Approximately 20 weeks later, the aorta and large vessels extending from the aortic arch to femoral bifurcation were isolated, stained with oil red O, and the extent of atherosclerotic plaques quantified. Negative controls included the use of an irrelevant rAAV, untransduced transplants and unmanipulated mice. Positive controls comprised wild type B6 bone marrow transplants into ApoE-/- mice. Although all ApoA-1 Milano treated groups showed marked reductions in plaque formation, transplantation with rAAV/Apo A-1 Milano-transduced marrow resulted in a significant 60-70% reduction in aortic atherosclerotic plaque formation. In contrast, the groups treated by intramuscular injection of rAAV-ApoA-1 Milano showed a 38-46% reduction in plaque formation as compared with controls. These results suggest that transplantation of rAAV-Apo A-1 Milano transduced bone marrow cells may provide a novel and efficient strategy for controlling the development of atherosclerosis.

56. Long-Term Correction of Murine Lipoprotein Lipase Deficiency by Single Intramuscular Administration of AAV1-LPL^{S447X}

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Lipoprotein lipase (LPL) plays a key role in triglyceride (TG) metabolism by hydrolyzing TG and mediating the uptake of TG-rich lipoproteins from blood plasma. In humans, LPL deficiency results in high TG and low high-density lipoprotein cholesterol (HDL-c) levels, chronic abdominal pain, and can cause life-threatening pancreatitis. In contrast, a natural LPL variant, LPL^{S447X}, is associated with a beneficial lipid profile.

Our aim is to develop a gene therapy to treat LPL deficient patients. In this study, we investigated the efficacy of a recombinant adeno-associated virus (AAV) vector expressing LPL^{S447X} to overcome hypertriglyceridemia in a murine model of LPL deficiency. In mice, complete LPL deficiency (LPL-/-) is lethal. However, this can be overcome at birth by intramuscular (IM) injection of adenovirus transiently expressing the human LPL^{S447X} variant. Rescued LPL -/- mice exhibit extremely high TG levels (200 fold higher than normal, >8000 mg/dl) and low HDL (10% of normal) by adulthood and were used in this study. A recombinant AAV2 vector was constructed containing the LPL^{S447X} cDNA driven by the CMV promoter and was pseudotyped with AAV1 capsids (AAV1-LPL^{S447X}). LPL -/- mice were injected IM in 4-40 sites with $5\text{x}10^{10}$ - $5\text{x}10^{11}$ genome copies (gc) AAV1-LPL^{S447X}, control vector AAV1-GFP, or PBS control. While lipid profiles were unchanged by AAV1-GFP or PBS control, AAV1-LPL^{S447X} proved an effective treatment. hLPL protein and LPL activity levels increased significantly in mice that received $5\text{x}10^{11}$ gc AAV1-LPL^{S447X} in 36 sites or $4\text{x}10^{11}$ gc in 40 sites .This resulted in a complete resolution of visible hyperlipidemia, detected as early as one week after injection (figure).TG were reduced up to 99% to near normal levels (around 170 mg/dl). We also observed a 3-8 fold increase in HDL-c and 12-19 fold decrease in total cholesterol, further confirming the beneficial effects of our vector. Other than a 1 week delay in disease resolution, 10 fold fewer injection sites gave similar results. A 10 fold lower dose showed intermediate improvements (up to 77% reduction in TG, to 2350 mg/dl). These effects have now persisted for 12 months.

Thus, we conclude that AAV mediated LPL^{S447X} expression in muscle is an effective therapy to provide long-term correction of hypertriglyceridemia in LPL deficient mice and holds promise for the treatment of human LPL deficiency. Experiments have been initiated in a feline model of LPL deficiency that closely resembles the human disease, to further evaluate the efficacy of our AAV1-LPL^{S447X} vector in a larger animal model.

EXHIBIT A